enced only the direction of change in the subsequent IFI, a pair of flashes being delivered every 1.5 seconds whether the subject responded or not. In the absence of a response the servomechanism continued to step in the same direction. Only a change in response (from right key to left key, or vice versa) would cause a reversal in the direction of alteration of IFI size. To control for hand-preference effects, the meaning of right and left keys was arbitrarily assigned to each subject, three of the subjects being instructed to use the right key to indicate "one" and the other three subjects being instructed to use the right key to indicate "two flashes." The controlling circuitry was altered according to whichever instructions were given, so that the records for all subjects were collected in similar form. There was no evidence that handedness biased the results in any way.

Each subject was tested and retested until he produced three sessions on three different days containing at least one drowsy episode in each session. The records for these days were exhaustively analyzed (not selectively sampled) in the following manner. Each separation threshold report (a response initiating a change in direction of the servomotor) was assigned a voltage score based on the average rate of voltage integration during the 60 seconds prior to the response. (A shorter voltage integration epoch of, say, 10 seconds can be used, but greater variability will be observed in the results; instantaneous voltage does not predict this type of performance as well as an average voltage derived from a longer sample. Some compromise must be made in order to avoid the variability that attends voltage measurements based on epochs of too short duration and to avoid serious loss of resolution of changes associated with real time if the epochs are too long.) The voltage scores expressed as percentages of the subject's alert voltage were then classified according to the number of milliseconds the corresponding threshold measurement lay above the subject's normal alert threshold, that is, according to the number of milliseconds of increase in the delay between flashes required to reach the separation threshold. This stabilization of the voltage scale in percentage units of the alert alpha voltage and the stabilization of the threshold measurements in units of IFI increase above the alert separation threshold facilitates the comparison and collation of the data from the various subjects.

Thresholds could not be assessed in the present arrangement beyond the range provided for in the mechanism. If a subject tracked to either limit and ceased to deliver any key taps for 60 seconds or more, it was assumed that he was "asleep" in the sense that he had lost effective contact with the testing situation, and therefore scoring was discontinued until he had returned to active tracking for 5 minutes or until he had resumed oscillatory tracking in the alert threshold range. This exclusion was necessary to avoid artifacts in the data attributable chiefly to inertia in the tracking system; if, for example, the subject recovered abruptly from a "lapse" in responding it would take some time before the servo-record actually reflected the subject's discriminative capacity.

The analyses are summarized in Fig. 1, which contains the data both for individual subjects and for the subjects collectively. Each of the individual records shows essentially the same relations found in the composite record: as alpha voltage declines from the normal alert level there is a rise in the separation threshold. What is of considerable interest is the fact that relatively small losses of alpha voltage are associated with rather sizeable increments in separation thresholds.

The present results are not attributable to motor failure to respond because all values are restricted to the occurrence of actual responses. In addition, the subjects were usually making key responses (in one direction only) for some minutes after they had tracked to the limit of the servomechanism. The primary loss, then, was not of motor capacity but rather of discriminative capacity.

These findings, taken in conjunction with the previous study on time estimation (*1*), indicate that great caution is required in controlling for the level of alertness in the measurement of psychological thresholds and in the assessment of performance, especially in cases where the procedures employed in the study tend to encourage drowsiness in subjects because of isolation, immobilization, and boredom. The absence of adequate controls on level of alertness renders the interpretation of such studies extremely problematical.

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6. Harvard cumulative recorder, Ralph Gerbrands Co., Arlington, Mass.
7. Photo-stimulator, model PS 2, Grass Instrument Co., Quincy, Mass.
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Inhibitory and Facilitatory Effect of Two Related Peptides on Extinction of Avoidance Behavior

Abstract. *The polypeptide chain which constitutes the first ten amino acids of the ACTH molecule inhibits extinction of a shuttle-box avoidance response. If the phenylalanine molecule in the 7th position of this peptide is replaced by its dextrorotatory form, extinction is facilitated.*

During studies on the inhibitory effects of adrenocorticotrophic hormone (ACTH) and related peptides on extinction of an avoidance response (*1*), we found that the polypeptide containing the first ten amino acids of ACTH, but in which the phenylalanine in the 7th position had been replaced by the dextrorotatory form [ACTH 1-10 (7-D-Phe)], exhibited an opposite effect.

Twenty-nine albino rats (male, 110 to 130 g) were conditioned in a shuttle-box divided into two equal compartments by a 5-cm barrier to avoid an electric shock (*2*). The floor was made of a grid through which an electric

Table 1. Amino acid sequence of ACTH β 1-24, α-MSH (melanocyte-stimulating hormone), and ACTH 1-10.

ACTH β 1-24	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro
α-MSH	CH_3CO-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly-Lys-Pro-Val
ACTH 1-10	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Try-Gly

shock (25 volts, 1.8 ma alternating current, 50 cy/sec) could be applied.

A buzzer (conditioning stimulus) was rung for 5 seconds before a shock (unconditioning stimulus) was given. The shock was terminated as soon as the rat had crossed the barrier. Rats were placed in the shuttle-box 60 seconds before the first trial of each session. Ten trials were given daily with a predetermined sequence of intervals between trials varying from 40 to 80 seconds. The average interval was 60 seconds. As soon as the animals reached the criterion for being conditioned (24 or more avoidances on each of three consecutive days), extinction trials were run with the same schedule as that used during acquisition trials. The conditioning stimulus was terminated after 5 seconds when the rat had not performed a response, and it was not followed by the unconditioning stimulus of shock. Of the 29 rats used, 27 achieved the conditioning criterion. These were randomly treated with either ACTH 1-10 (7-L-Phe) [adrenocorticotrophic hormone with the levorotatory form of phenylalanine in the 7th position (*3*)], ACTH 1-10 (7-D-Phe) or a placebo. The amino acid sequences of ACTH β 1-24, α-MSH (melanocyte-stimulating hormone), and ACTH 1-10 are presented in Table 1.

Immediately after the last session of training, 10 μg of a long-acting zinc phosphate preparation of one of the peptides (*1*) or 0.5 ml of the zinc phosphate complex, as the placebo, was administered subcutaneously to the animal. Treatment was given every other day during the extinction period.

ACTH 1-10 (7-L-Phe) delayed extinction of the avoidance response (Fig. 1). In contrast, the D-form facilitated extinction. The number of conditioned avoidance responses (CAR) during the 7-day period of extinction of the group given the placebo was 45 $\pm$ 2.6, that of the group treated with ACTH 1-10 (7-L-Phe) was 61 $\pm$ 1.0 and that of the group treated with ACTH 1-10 (7-D-Phe) was 10 $\pm$ 2.1. Reconditioning of the animals treated with the D-form of the peptide after the 7th day of extinction resulted in complete reappearance of the conditioned avoidance response, despite continuation of treatment. These animals scored 25 $\pm$ 0.4 conditioned avoidance responses in 30 trials during the reconditioning period.

Since ACTH (*1, 2, 4*) α-MSH (*1, 4*), and ACTH 1-10 (*1*) inhibit extinction of the avoidance response, it is reasonable to assume that an amino acid sequence common to these peptides is responsible for the behavioral effect. Accordingly, the opposite effect of the D-form of the peptide may be caused by direct, antagonistic action to such pituitary hormones as ACTH and α- and β-MSH.

The effect of ACTH 1-10 (7-D-Phe) on conditioning was studied in animals in which the pituitary was removed. Thirty-three rats were trained to acquire the conditioned avoidance response in the shuttle-box as described above. After having achieved the criterion for conditioning, animals were either hypophysectomized (transauricular approach) or subjected to sham-operations. The animals were allowed to recover for 2 weeks during which they were treated with 0.25 mg cortisone acetate, 0.2 mg testosterone propionate, and 0.01 mg L-thyroxine injected subcutaneously every second day. Then they were retrained until the criterion for conditioning was again reached. Extinction was studied for the next 6 days. Fifteen sham-operated and 18 hypophysectomized rats were treated with the D-form of the peptide or the placebo. Extinction of the conditioned avoidance response was facilitated in the hypophysectomized rat in a fashion similar to that found in sham-operated animals (Fig. 2). Extinction was even faster in the former group

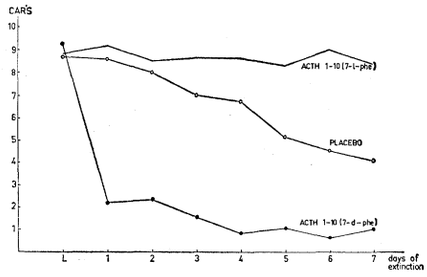

Fig. 1. Effects of ACTH 1-10 (7-L-Phe), ACTH 1-10 (7-D-Phe), and placebo on the extinction of a shuttle-box avoidance response.

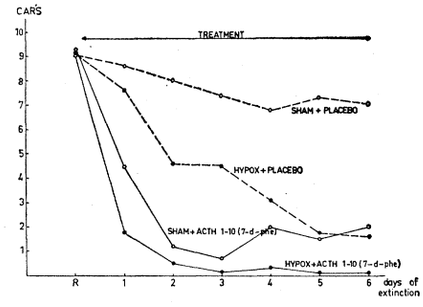

Fig. 2. Effect of ACTH 1-10 (7-D-Phe) and placebo on extinction of a shuttle-box avoidance response in sham-operated and hypophysectomized rats.

than in the latter. Whereas hypophysectomized rats treated with ACTH 1-10 (7-D-Phe) scored 3 $\pm$ 0.6 CAR, sham-operated animals made 12 $\pm$ 1.4 CAR. Thus, the facilitative effect of the D-form of the peptide on extinction of the CAR cannot be explained by assuming that there is a direct antagonism between it and the structurally related L-form of the peptides of pituitary origin.

To assess whether alterations in motor and sensory function could explain the marked behavioral effect of the two peptides, escape behavior of 18 rats under chronic treatment was studied in a (2.4 m long) runway equipped with a grid floor (*5*). The

Table 2. The effect of long-acting ACTH 1-10 (7-L-Phe), ACTH 1-10 (7-D-Phe), and zinc phosphate complex on behavior in an open-field. The mean numbers plus or minus the standard errors of the means of the fecal boluses, crossings, rearings, and grooming movements are given.

Treatment	Rats (No.)	Fecal boluses (No.)	Ambulation (No. of crossings)	Rearing (No.)	Grooming movements (No.)
Placebo 0.5 ml (zinc phosphate complex)	11	3.2 $\pm$ 0.5	46.2 $\pm$ 6.3	16.3 $\pm$ 2.7	7.2 $\pm$ 1.8
ACTH 1-10 (7-L-Phe) 10 μg	12	3.7 $\pm$ 0.5	46.2 $\pm$ 4.6	13.2 $\pm$ 2.9	12.0 $\pm$ 3.2
ACTH 1-10 (7-D-Phe) 10 μg	12	3.6 $\pm$ 0.5	56.0 $\pm$ 5.5	18.7 $\pm$ 2.1	10.2 $\pm$ 2.6

speed with which a rat tried to escape an unavoidable shock (50 volts, 0.25 ma) served as an index of pain-motivated escape behavior. There was no difference between the escape speed of the groups treated with the two peptides and that of the group given the placebo (Fig. 3). This finding indicates that the behavioral effect of the two peptides was not attributable to alterations in motor and sensory capacities or to changes in escape motivation.

The effects of ACTH fragments on the behavior of 35 male rats was studied in an open-field test 18 hours after each had been given a single injection of either the D-form or the L-form of the peptide or a placebo. The number of fecal boluses, the number of crossings of squares (10 by 10 cm) on the floor of the open-field, any behavior during which the rats were standing on their hind legs (rearing) and the number of washings, lickings, or scratchings of their own body (grooming) were recorded for 3 minutes. There were no differences between the numbers of boluses, explorations, rearings, groomings, or crossings of the rats treated with ACTH 1-10 (7-L-Phe) and those of rats treated with ACTH 1-10 (7-D-Phe).

We have shown that two closely related peptides, differing only in the rotation of the phenylalanine in the 7th position of the amino acid chain 1 to 10 of the ACTH molecule, alter the rate of extinction of a conditioned avoidance response in opposite ways. The site of action of these peptides must be sought in the central nervous system. Whether they act via autonomic nervous transmission or by a direct influence on nerve cells is not known.

The importance of the role of peptides on behavior has been demonstrated by the observation that transfer of conditioned responses from trained to untrained rats can be induced by the administration of brain extracts from trained rats to untrained ones (*6*). The information bearing molecule appears to be a polypeptide with a molecular weight between 1000 and 5000 (*6*) which is well within the range of the molecular weight of the peptides used in our study.

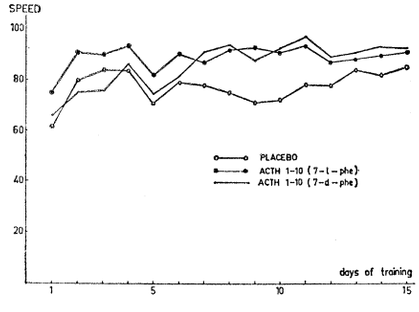

Fig. 3. Effect of ACTH 1-10 (7-L-Phe), ACTH 1-10 (7-D-Phe), and placebo on escape speed (reciprocal of latency) in a runway alley.

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Semiconductor Detectors

The report on the "Application of high-resolution semiconductor detectors in x-ray emission spectrography" by Bowman *et al.* (*1*) must be exciting to everyone who uses x-ray spectrography for chemical analysis. As Bowman *et al.* point out, when the resolution of the x-ray detector is good enough, the diffractor may be omitted from the analytical system with a tremendous gain in simplicity and absolute sensitivity. However, their report seems to leave the impression that the semiconductor detectors have opened up this possibility for the first time. In fact, this method has been used for many years, usually under the name "nondispersive analysis," with the gas-filled proportional counter serving as the detector. For example, nondispersive analysis has already been implemented with gas counters for the various means of excitation mentioned in the report: radioactive sources (*2*), x-ray tubes (*3*, *4*), and electron probes (*5*).

The resolution of gas counters leaves much to be desired, so that analysts have been looking forward to the time when improved semiconductor units would offer substantially better performance even at relatively low quantum energies. It seems likely that the semiconductors will ultimately be quite superior. But there remains the important practical question of the relative merits of the different detectors at the present moment.

A few remarks may help to begin a comparison. For nickel K x-rays, Bowman *et al.* quote a full width at half maximum of 1.3 kev, which is similar to the performance of gas counters. At low quantum energies the gas counters are presently favored by their lesser noise. At much higher energies the semiconductors seem clearly superior in both efficiency and resolution, but in this range one should also consider scintillation counters, which have poorer resolution but are very convenient. One must bear in mind that the capacity to resolve close x-ray lines as visibly separate peaks is no absolute criterion; there may still be severe problems of quantitation at low concentrations, while on the other hand, accurate quantitative analysis may be possible when the pulse spectra are not visibly separate (*4*, 5).

However, the comparison really hinges on factors which are unfamiliar to most analysts because advanced semiconductor technology is not widely known. While gas counters are intrinsically bulky, the semiconductors must be cooled; which system can be maneuvered in general to intercept a larger fraction of the radiation from the specimen? How do the detectors and their electronics compare in reliability, convenience, and cost? I hope Bowman *et al.* will comment on these points, to help us judge if the time has yet come to throw away the gas counters.

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